

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Biofilms: A Challenge to Medical Fraternity in Infection Control

Silpi Basak, Monali N. Rajurkar, Ruchita O. Attal and Sanjay Kumar Mallick

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55649>

1. Introduction

Microbes have been characterized as planktonic, free-floating single cells. The morphological and physiological properties of microbes have been described as they grow in nutritionally rich culture media. Earlier very little thought have been given how microbes survive in the environment. But, the fact is, in natural environment, microbes are commonly found to be attached to surfaces as biofilms. Hence, the formation of surface attached microbial cells known as biofilms open a new horizon to study the micro-organisms.

Automatically, the question arises, “What is biofilm?” According to the recent definition, Biofilms can be defined as sessile communities of microbial cells irreversibly attached to a surface or interface or to each other which are embedded in a self produced matrix of extracellular polymeric biomolecules and are physiologically different from planktonic cells with respect to growth rate and gene transcription [1]. While studying *Pseudomonas aeruginosa* Davis and Geesay have shown that gene *algC* controlling phosphomannomutase involved in alginate (exopolysaccharide) synthesis is upregulated within 15 minutes of adhesion to a solid surface [2].

Biofilms are ubiquitous. They can be present on any surface – biotic or abiotic. Biofilms can be found on ship hulls, dairy and petroleum pipeline and rocks or pebbles at the bottom of streams or rivers. They can grow in hot acidic pools in Yellowstone National Park (USA) and on glaciers in Antarctica. Biofilms can form anywhere with easy access to water e.g. on tiles of floor, kitchen platform or clogged sink etc. They are also found on plants and can remain symbiotically or cause crop diseases like citrus canker, Pierce’s disease of grapes etc [3]. Fossilised biofilms with 3.5 billion years are among the oldest records of life on earth [4]. Biofilms are also

associated with biocorrosion of metals (microbiologically influenced corrosion, i.e. MIC) which affect kinetics of cathodic and/or anodic reactions [5]. Biofilms can also grow in contact lenses, biomedical implants and transcutaneous devices.

Nearly every species of microorganisms e.g. bacteria, fungi, algae and protozoa have mechanisms to adhere to surfaces and to each other. It has been found that over 90% of all bacteria live in biofilms. Biofilms can be formed by single species of microorganism or by multiple species of bacteria, fungi, protozoa etc. Mixed species biofilms predominate in environment. Single species biofilm usually exist in a variety of infections and on medical implants and are the focus of current research [6].

Study of biofilm began when it was discovered that in natural aquatic system bacteria predominantly remain attached to surfaces [7]. The first recorded observation of biofilm was presented by Henrici in 1933 as 'it is quite evident that for the most part water bacteria are not free floating organisms, but grow upon submerged surfaces' [8]. The fouling of ship hulls by microbes in marine environment was already known to mankind. Hence, the study of biofilm has been started with marine bacteria, followed by fresh water microbial ecosystem and formation of biofilm on surface of eukaryotic tissue.

In early part of 20th Century it was difficult to observe biofilm as electron microscopy required complete dehydration of highly hydrated biofilm matrices and light microscopy was badly distorted by out-of-focus effects [1]. Though Confocal Laser Scanning Microscope (CLSM) was invented in 1950s it was never used to study bacteria. CLSM produces optical slices of complex structures, so out of focus effects are removed and it requires no sample preparations, so living microorganisms can be observed if fluorescent dye is introduced to observe the cells [1]. Hence, the modern biofilm era began with the use of Confocal Laser Scanning Microscope (CLSM) which showed the image of biofilm as sessile microbial cells embedded in matrix interspersed between open water channels [9].

The development of biofilm is a 5 stage process – 1) reversible attachment 2) irreversible attachment 3) early development 4) maturation 5) detachment or dispersal of cells. When the microbial cell reaches very close to a surface (<1nm), the initial attachment depends upon the total attractive or repulsive forces between two surfaces. These forces include electrostatic and hydrophobic interactions, steric hindrance, van der Waals forces etc. Probably hydrophobic interactions play important role in primary adhesion [10]. The second stage of irreversible attachment employs molecular binding between specific adhesins and the surfaces [11].

The factors controlling biofilm formation are: i) recognition of attachment sites on a surface ii) nutritional cues iii) change of pH and temperature iv) exposure to antibiotics, chemical biocides, and host defense mechanisms e.g. complement system etc.

The gene expression in biofilm cells differ from planktonic cells and by 2D gel electrophoresis it had been found that in mature biofilm of *Pseudomonas aeruginosa* >300 proteins were detectable that were undetectable in planktonic cells [12].

During colonisation, microbial cells communicate via quorum sensing. In mature biofilm quorum sensing regulates formation of channels and pillar like structure for nutrient delivery.

Microbial cells in biofilms undergo cell density-dependent gene regulation i.e. quorum sensing and thus coordinate through signalling molecules called autoinducers. Autoinducers increase in concentration as a function of cell density [13]. Usually Gram positive bacteria use processed oligopeptides to communicate, whereas Gram negative bacteria use N- acyl homoserine lactones (AHLs) as autoinducers [14]. The widespread AI-2 quorum-sensing system is found in several, Gram positive and Gram negative bacteria also [15]. For acyl-HSL quorum-sensing, an enzyme belong to Lux I family is required for synthesis of signal from cellular metabolites [16]. For AI-2 quorum-sensing system which has been implicated in interspecies communication, the synthesis of signalling molecule is directed by the Lux S gene product [17]. The *ahyR*/I acyl- HSL quorum sensing system of *Aeromonas hydrophila* has been shown to be required for biofilm maturation [12]. Similarly the Lux S type quorum sensing system in *Streptococcus mutans* is also involved in biofilm development. Lux S system of *Salmonella enterica* serovar Typhimurium is required for biofilm formation on human gallstones [18].

Duenne described biofilm architecture as underwater coral reef with pyramid or mushroom shaped projections from the surface and channels and caverns running through out [19]. Using CLSM, Lawrence et al has shown that *Pseudomonas* biofilms were more tightly packed at the surface and less dense near the periphery whereas *Vibrio parahaemolyticus* biofilms show greatest cell density near the periphery [20].

The adherent cells in a biofilm are embedded with a self produced matrix of extracellular polymeric biomolecules. 97% of a biofilm matrix is water. A complex of secreted polymers, absorbed nutrients and metabolites, cell lysis product and even particulate materials from the surrounding environment can form matrix. Actually the matrix surrounds, anchors and protects surface-bound microbes. The matrix actually prevent the access of antimicrobials and disinfectants and confer protection against environmental stresses such as UV radiation, pH shifts, osmotic shock and dessication [21].

Besides microbial cells all major classes of macromolecules i.e. proteins, polysaccharides, nucleic acids can be observed within a biofilm. Even transformation, transduction and conjugation result in gene transfer amongst the cells in biofilm.

Biofilms are formed by many bacterial species of medical importance e.g. *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococci*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *E.coli* O157:H7, *Neisseria gonorrhoeae*, *Vibrio cholerae*, *Nontuberculous mycobacteria* (NTM) etc [6]. Amongst fungi - *Candida albicans* can usually form biofilm [22]. The two most intensely studied biofilms are produced by: *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

1.1. Biofilms and human disease

The microbial biofilm has received much attention recently because biofilm mode of growth may be the key factor in persistent or chronic infections. The biofilms can act as nidus of acute infections and the microbial cells from biofilm are released at any one time during chronic infection [23]. Clinicians are very much concerned about the fact that it is really difficult to eradicate biofilm bacteria with antibiotics. Even in immunocompetent host the biofilm growth are rarely resolved by host's immune system as antigen may be hidden and key ligands may

be repressed [24]. Biofilms are associated with kidney stones of infective origin, formation of dental plaques, infections in cystic fibrosis, infections of permanent indwelling devices such as joint prosthesis & heart valves, intrauterine devices (IUDs) and urinary catheters etc [25].

However in many chronic infections both the biofilm and planktonic growth may coexist. Parsek and Singh in 2003 have proposed few criteria to define the role of biofilms in human diseases [12]: a) the causative bacteria are surface associated b) examination of infected tissue shows bacteria living in microcolonies and embedded in extracellular matrix c) infection is usually confined to a particular site and dissemination occurs as a secondary phenomenon d) the infection is difficult to eradicate with antibiotics though the causative bacteria are susceptible to that antibiotics in planktonic state.

1.1.1. Infection-related kidney stones

15-20% of kidney stones occur in the setting of urinary tract infections. Infact, infection stones are produced by interplay between infecting bacteria and mineral substrates derived from urine resulting in formation of a complex biofilm. Microscopic analysis of stone has revealed that bacteria are organized in microcolonies and surrounded by an anionic matrix composed of both polysaccharides and crystallized minerals [26]. It requires an alkaline environment to decrease solubility of phosphate, increased concentration of NH_4^+ for struvite and CO_3^{2-} for carbon apatite formation as these are major constituents of this type of stone. The normal urine is not saturated with struvite and carbon apatite. The alkaline pH of urine occurs in infection with urease producing organisms like *Proteus*, *Providencia*, *Klebsiella* and *Pseudomonas* species. It is hypothesized that biofilms provide localized and concentrated urease activity to form stones [26].

1.1.2. Bacterial endocarditis

The primary lesion in endocarditis is due to vegetation (valve biofilm), which is composed mainly of bacteria and their products, platelets and fibrin derived from circulation with the damaged endothelial surface as substratum. Durack in 1975, developed nonbacterial thrombotic endocarditis by leaving a polyethylene catheter in contact with aortic valve of a rabbit and showed how bacterial microcolonies were formed within 24 hours [27].

1.1.3. Airway infections in cystic fibrosis

Cystic fibrosis (CF), a common inherited disease of lower respiratory tract is caused by mutation in the gene which encodes Cystic fibrosis transmembrane regulator protein (CFTR). CFTR functions as a chloride ion channel protein [1]. Chloride ion transport is severely impaired when CFTR is defective in CF patients, resulting in hyperviscous mucus. Initially CF patients suffer from intermittent respiratory infections but in late stage permanent infection with *P. aeruginosa* occurs. It has been found that even with higher antibiotics given parenterally *P. aeruginosa* could not be eradicated from sputum of CF patients in the late stage and it may persist for the rest of the patient's life. In permanent infection phase of CF patients, *P. aeruginosa* biofilm may be found in airways. Another

interesting finding is emergence of *P. aeruginosa* with mucoid phenotype in late stage CF patients [28]. This mucoid material is a polysaccharide i.e. alginate which probably prevent antibody coating and opsonic phagocytosis. In fact, biofilm protects *P. aeruginosa* from antimicrobials and host defenses. Genetic fingerprinting studies show same strain of *P. aeruginosa* can persist in CF patients for decades leading to chronic inflammation and decline in lung function and ultimately respiratory failure [29].

1.1.4. Endodontics

Biofilms also play a major role in causing dental caries, gingivitis, periodontitis, apical periodontitis etc [30]. The anatomical complexities in root canal system provide favourable condition for biofilm formation, which is actually initiated by invasion of pulp chamber by oral flora after tissue breakdown. Facultative or strict anaerobes are more frequently associated than aerobic microorganisms. *Porphyromonas gingivalis* is the primary agent responsible for periodontitis [31]. Endodontic biofilm can be—i) intracanal, ii) extraradicular, iii) periapical and iv) foreign body centered. Foreign body centered biofilm is a major complication associated with prosthesis and implant supported prosthesis [32].

1.1.5. other conditions

Similarly during acute phase of osteomyelitis, microscopical examination have shown biofilm formation on infected bone surfaces [33]. In chronic prostatitis, adherent bacterial colonies on the surface of prostatic duct have been observed on microscopical studies, even in culture negative cases [34].

1.1.6. Indwelling medical devices

Biofilms can develop on indwelling medical devices like prosthetic heart valve, pacemakers, central venous catheter, urinary catheter, contact lenses, intrauterine devices etc. and can cause persistent infections which are usually lethal. Scanning electron microscopy clearly shows biofilm formation at the tip of urinary catheter kept for 7 days. On medical devices, biofilms are most commonly formed by coagulase negative Staphylococci (CoNS) especially *S. epidermidis* followed by *S. aureus*, Enterococci, *Pseudomonas aeruginosa* etc.

Biofilms can develop on both types of contact lenses i.e. soft and hard and also on contact lens storage cases. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E.coli*, *Candida* species can adhere to contact lenses [35]. Evidence of biofilm on contact lenses and it's storage cases have been reported from patients with microbial keratitis [36]. The rate of prosthetic valve endocarditis (PVE) range from 0.5% to 4% [37]. Coagulase negative Staphylococci are the commonest early colonizers after surgical implantation of prosthetic valve whereas *Streptococcus viridans* most commonly colonize during late PVE (i.e. 12 months following valve replacement) [38]. Though *S. aureus*, Gram negative coccobacilli or fungi may also be responsible for PVE.

Infection with central venous catheter is a quite common device related infection. Biofilms have been shown by CLSM to be present outside the catheter or inner lumen [34].

In *S. epidermidis* biofilm initial adherence is by polysaccharide adhesin (PSA) and accumulation of cells is due to production of polysaccharide intercellular adhesin (PIA). PIA is encoded by *ica* (intercellular adhesin) operon *ica* ADBC [39]. The *icaR* gene regulates *ica* operon. Production of PIA is also subject to ON - OFF switching (phase variation). Majority of clinical isolates of *S. aureus* also possess *ica* structural genes [40].

1.1.7. Health care Associated Infections (HAI) and biofilm

Catheter Associated Urinary Tract Infection (CA-UTI) is the commonest (>40%) HAI [41]. Nosocomial bacteriuria or candiduria develops in 25% of patients having urinary catheter for >7 days with a daily risk of 5% [42]. Most infected urinary catheters are covered by a thick biofilm containing infecting microorganisms. A biofilm forms intraluminally or extraluminally or both ways.

With the increasing use of vascular access devices, catheter related bloodstream infection (CR-BSI), septic thrombophlebitis, endocarditis and other metastatic infections e.g. lung abscess, osteomyelitis and endophthalmitis etc. are also increasing. In the United States out of 5 million Central Venous catheters used each year, 3-8% lead to BSI [43]. The initiation of catheter colonization occurs with the formation of a biofilm in the catheter lumen. Moreover, the resistance levels of biofilm associated organisms may be much higher than those of planktonic organisms [44]. After stoppage of antimicrobial therapy, the biofilm associated organisms resurge and cause another clinical infection. A recent approach to reduce CR-BSI is bundles of preventive measures, which means a group of preventive measures, when executed together, result in better outcomes than when implemented alone [45]. This included handwashing, using full barrier precautions during insertion of central venous line, cleaning the skin with chlorhexidine. The femoral site should be avoided if possible and catheters should be removed as early as possible.

Hospital acquired pneumonias are the second most common cause of HAI and has the highest morbidity and mortality of all HAIs [46]. The initial step in pathogenesis of HAP is colonization of patient's oropharynx with resistant hospital pathogen. The endotracheal tube lumen is a nidus for the growth of bacteria within the biofilm. Hand washing and Personal protective equipment (PPE) must be used to reduce the incidence of HAP/ Ventilator associated pneumonia (VAP).

1.1.8. Resistance of biofilm to antimicrobials and disinfectants

It has been observed that biofilms are not easily eradicated even by cidal antimicrobials, quaternary ammonium compounds, halogens and halogen release agents. The crux of the problem is the presence of persisters within the biofilms that can rebound when antibiotic concentration falls. The causes are multifactorial – i) restricted penetration of antimicrobials within the biofilm architecture, ii) decreased growth rate of bacterial cells forming the biofilm, iii) expression of resistance gene by the bacterial cells within the biofilm etc [47]. Restricted penetration of antimicrobials may occur as negatively charged exopolysaccharide restrict permeation of positively charged antibiotics e.g. Aminoglycoside and exopolymer matrix also

restrict diffusion of antimicrobial within the biofilm. Synergy between retarded diffusion and degradation by enzymes (e.g. β -lactamase) also provide effective resistance to antimicrobials. Fluoroquinolones are very effective in stopping the growth of a biofilm but restricted diffusion can protect the microbial cells within the biofilm [48]. All antimicrobials are more effective in killing rapidly growing cells. Penicillin & ampicillin do not kill non-growing cells as rate of killing is directly proportional to rate of growth for these two antibiotics. Even cephalosporins, aminoglycosides & fluroquinolones can kill rapidly dividing cells more effectively. Multiple drug resistance (MDR) pumps may play a role in biofilm resistance at low antibiotic concentration. Sometimes unknown MDR pumps might be over expressed in biofilm e.g. for chloramphenicol in *E. coli* biofilm. Moreover, the biofilms increase the opportunity of gene transfer between the microorganisms and can convert a previously avirulent commensal organism to a highly virulent pathogen. The enhanced efficiency of gene transfer in biofilms also facilitates the spread of antibiotic resistance and virulence factors [49]. Though most of the research works deals with single species biofilms, multispecies biofilm amongst different bacteria and interkingdom biofilms between fungus *Candida albicans* and various bacterial species are also gaining importance in causing different diseases [50]. Biofilm formation is a major virulence factor for *Candida albicans* and *Candida* biofilms are difficult to eradicate due to their high resistance to antifungals. A recent study has reported that within the biofilm *Staphylococcus aureus* was attached uniquely with the pseudohyphae of *Candida albicans*. This synergistic interaction resulted in differential protein expressions which are actually virulence factors for *Staphylococcus aureus*. This indicate *C. albicans* may enhance *S.aureus* pathogenesis [51]. Recently it has been reported that co-existence of *S.aureus* and *C.albicans* in a biofilm resulted in increased Vancomycin resistance in *S.aureus* [52] However antagonistic interaction has been reported between *Pseudomonas aeruginosa* and *Candida albicans* [53].

It is not possible to detect the antimicrobial resistance of biofilms by conventional methods of disc diffusion and broth microdilution as per CLSI guideline because these methods are only meant for planktonic cells.

1.1.9. Biofilms and altruism

Biofilms are like small cities and encourage altruism. Microbial cells within biofilm often sacrifice their maximum growth rate to use the available community resources more efficiently. In a biofilm atleast some of the microbial cells experience nutrient limitation and exist in a slow growing state [54]. In this process while individual cells are disadvantaged, the microbial community as a whole is benefited. Hence, it is said that biofilms are the colonial way of life of microorganisms.

Detection of biofilms can be done by both phenotypic methods and genotypic methods. In phenotypic methods biofilms are detected by Congo red agar method(CRA), Plastic tube method(TM), Tissue culture plate method(TCP) and Confocal Laser Scanning Microscopy (CLSM). In genotypic method, usually Polymerase chain reaction (PCR) for amplification of microbial DNA, coding for biofilm formation is done. The phenotypic methods are easy and cheap compared to genotypic method.

Hence, the present study was undertaken to detect the biofilm producing organisms, isolated from different clinical specimens in our laboratory.

2. Material and methods

The present study was conducted from 2009 to 2012. A total number of 350 bacterial and 50 *Candida* strains were studied. The microbial strains were isolated from different clinical specimens like urine, blood, pus and wound swab, endotracheal aspirate, urinary catheter tip, central venous catheter tip etc. All the microbial strains were identified by conventional methods [55]. We used microtitre plate biofilm assay to detect microbial attachment to an abiotic surface [56].

Steps:

1. The microbial cells were grown in Brain heart infusion broth overnight.
2. On next day, the cultures were diluted 1:100 using the brain heart infusion broth.
3. 100µl of each diluted culture was inoculated into each of three wells in a microtiter plate which has not been tissue culture treated. The plates were covered by the lid and was incubated at optimal growth temperature [56] for 48 hours
4. Then the wells were washed twice to remove planktonic cells.
5. Microbial cells which were adhered to the wells were subsequently stained with crystal violet solution that allowed visualisation of the attachment pattern. 125 µl of 0.1% crystal violet solution was added to each well and stained for 10 minutes at room temperature.
6. The microtiter plates were shaken and the crystal violet solution was removed.
7. The plates were washed successively twice with distilled water. Any crystal violet that is not specifically staining the adherent microbial cells were removed by this washing step.
8. The microtiter plates were then inverted and tapped vigorously on tissue paper to remove any excess liquid.
9. The microtiter plates were then air dried. The dried microtiter plates may be stored at room temperature for several weeks.
10. This surface associated dye was solubilized by adding ethanol or any other solvent for semiquantitative assessment of biofilm formed. 200µl of 95% ethanol or other appropriate solvent [56] was then added to each stained well and was kept for 10 to 15 minutes
11. The contents of each well were mixed by pipetting and then 125µl of the crystal violet / ethanol solution from each well was transferred to a separate well of another 96 well microtiter plate maintaining the same sequence.

12. Then the optical density of each well containing 125µl solution was measured at a wavelength of 545nm in an ELISA reader. As each strain was put in triplicate the average of the three readings were taken.

The biofilm formation of different strains were classified in three groups according to the cut off OD. The cut off OD (OD_c) for the microtiter plate test was defined as three standard deviations above the mean OD of the negative control. Isolates were classified into four groups as nonadherent, weakly adherent, moderately adherent and strongly adherent according to Stepanovi et al [57].

3. Observation and results

Out of 350 bacterial strains studied, 90 were *Pseudomonas aeruginosa*, 80 were *E. coli*, 35 were *Klebsiella pneumoniae*, 80 were Coagulase positive *Staphylococci*, 30 were Coagulase negative 35 included *Proteus* sp(5), *Vibrio cholerae*(3), *Acinetobacter baumannii*(4), *Enterococcus* sp.(23). Out of 50 *Candida* strains 23 were *Candida albicans*, 16 were *Candida tropicalis*, 2 were *Candida dubliensis*, 6 were *Candida krusei* and 3 were *Candida glabrata*. Amongst 350 bacterial strains, 153(43.7%) and out of 50 *Candida* species 28(56%) were biofilm producers respectively. Amongst 50 *Candida* species, 11 (22%) were strong biofilm producers, and 6/11 (54.5%) were *Candida albicans*.

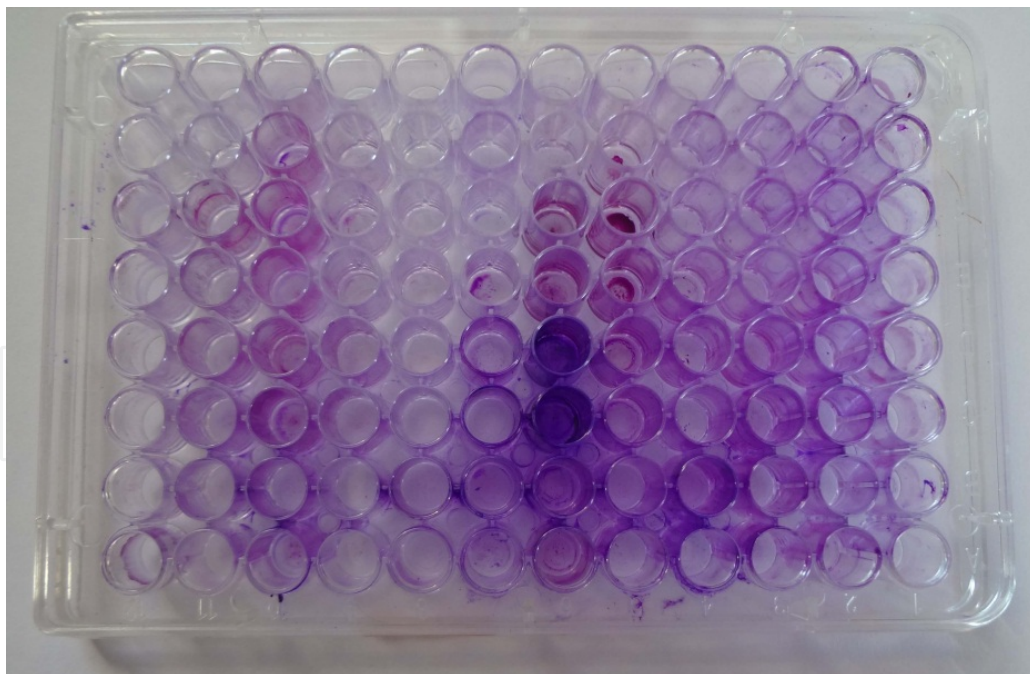


Figure 1. Microtitre plate biofilm assay for detection of microbial attachment

Maximum 65(72.2%) of *Pseudomonas aeruginosa* strains produced biofilms. 51(33.3%) biofilm producing bacterial strains were isolated from catheterized urine samples or patients having

other medical devices. 108(70.6%) bacterial strains producing biofilms were isolated from patients having chronic infections eg persistent or recurrent UTI, Chronic obstructive airway disease, cystic fibrosis etc.

In our study the cut off OD(ODc) was 0.003. The biofilm forming organisms are grouped into weak group (OD ≥ 0.003 to 0.006), moderate group (OD ≥ 0.006 to 0.012) and strong group (OD > 0.012).

Organisms	Newer β – lactamases producers							Non β – lactamase producers
	ESBL Only	AmpC Only	MBL Only	ESBL + AmpC	ESBL + MBL	AmpC + MBL	ESBL + AmpC +MBL	
P.aeruginosa [90]	10	14	10	23	-	-	-	33
Strongly adherent biofilm producing P.aeruginosa	7	6	4	11	-	-	-	5
E.coli [80]	10	7	4	38	1	-	1	19
Strongly adherent biofilm producing E.coli	6	4	2	20	1	-	-	1
Klebsiella pneumonia [35]	3	2	1	8	2	-	-	19
Strongly adherent biofilm producing K. pneumoniae	1	1	1	4	1	-	-	2
Proteus species [5]	3	1	-	1	-	-	-	-
Strongly adherent biofilm producing Proteus species	1	-	-	-	-	-	-	-
Acinetobacter baumani [4]	1	1	2	-	-	-	-	-
Strongly adherent biofilm producing Acinetobacter baumani	-	-	2	-	-	-	-	-

Table 1. Incidence of strong biofilm producers amongst newer β – lactamases producing strains

It was observed that out of 57 newer β – lactamases (Extended spectrum β – lactamases i.e. ESBL, Amp C β – lactamases and Metallobetalactamases i.e. MBL only and in combination)

producing *Pseudomonas aeruginosa* 28 (49.1%) were strongly adherent biofilm producers, compared to only 5/33 (15.1%) non β – lactamase producers. Amongst the 120 Enterobacteriaceae strains studied. 82 (68.3%) were newer β – lactamases producers, whereas 48/82 (58.5%) were strong biofilm producers and only 3/38 (7.9%) non β – lactamase producing strains were strong biofilm producers.

Organisms	Methicillin resistant	Methicillin sensitive
Coagulase positive Staphylococcus [80]	34	46
Strongly adherent biofilm producing Coagulase positive Staphylococcus	14	2
Coagulase negative Staphylococci (CONS) [30]	11	19
Strongly adherent biofilm producing Coagulase negative Staphylococci (CONS)	4	2

Table 2. Incidence of strong biofilm producing Methicillin resistant Staphylococcus strains.

Table 2 shows amongst the Methicillin Resistant Staphylococcus aureus (MRSA) strains, 14/34 (41.2%) and Methicillin Resistant Coagulase negative Staphylococci (MR – CONS) 4/11 (36.4%) were strong biofilm producers compared to 2/46 (4.3%) *Methicillin sensitive Staphylococcus aureus* (MSSA) and 2/19 (10.5%) Methicillin sensitive CONS.

Out of 23 Enterococcus species 13/23 (56.5%) were High level Aminoglycoside Resistant (HLAR) strains and it was also found that 8/13 (61.5%) HLAR strains were strong biofilm producers compared to only 2/10 (20%) of non HLAR strains.

4. Discussion

Our Hospital is a tertiary care centre in a rural setup. Though CLSM is the best phenotypic method, it could not be used as it is very costly. We did a pilot study with Staphylococci in 2008 and found 33% of Staphylococcus aureus and 44.7% of Coagulase Negative Staphylococci (CONS) were biofilm producers and amongst the 3 phenotypic methods tissue culture plate method gave the best results [58]. The present study correlated well with reports of other authors that Extended Spectrum β -Lactamase (ESBL) producing strains, Methicillin Resistant Staphylococcs aureus(MRSA) were more adherent to microtitre plate than Non ESBL and Non MRSA strains (Figure 2).

Lee et al in 2008 have also reported a positive correlation between biofilm formation and ESBL producing *Acinetobacter baumannii* [59]. Norouzi et al in 2010 have reported that in their study 14% ESBL producing *Pseudomonas aeruginosa* has formed strongly adherent biofilm compared to only 4% of non-ESBL producing *Pseudomonas aeruginosa* [60]. It has also been

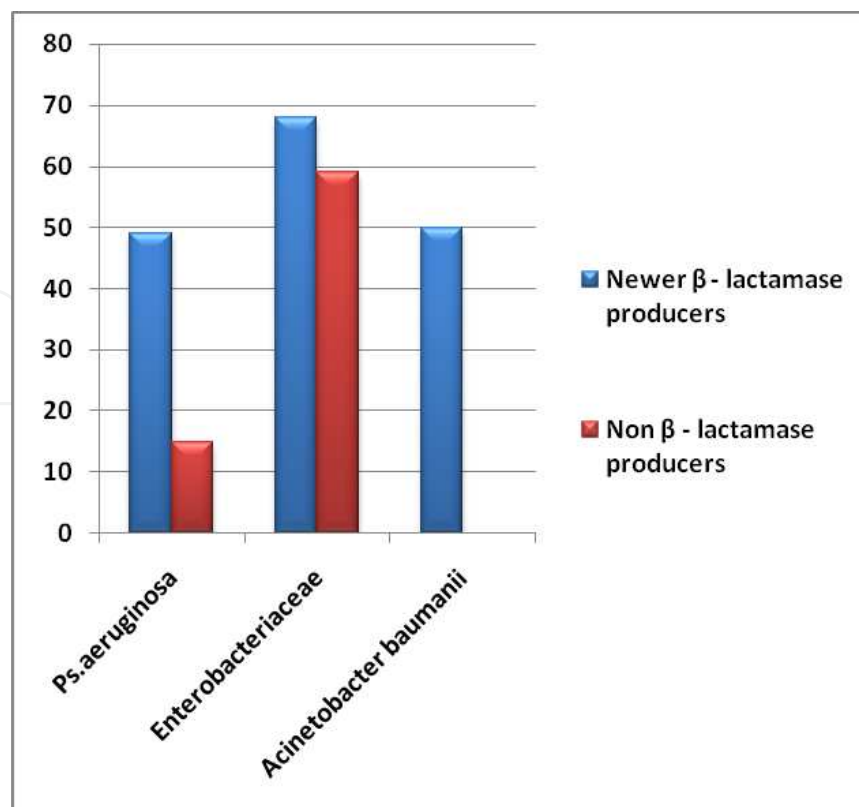


Figure 2. Strongly adherent biofilm producing strains (%) amongst newer β – lactamase producers and non β – lactamase producers

reported that, biofilm production was higher amongst MRSA strains as compared to Metthi-cillin sensitive *S.aureus* (MSSA) strains [61].

5. Treatment and control strategies

As far as the treatment of the persistent infection with medical device is concerned, the first step is to remove the infected indwelling medical device. Several control strategies have been proposed for biofilms e.g. systemic ciprofloxacin therapy in catheterized patients [1], latex catheter coated with silicone or silver hydrogel, catheter containing or use of antibiotics specially combination of Rifampicin and Minocyclin into material of indwelling catheters [62], Nitrofurazon coating, pretreatment of catheter surfaces with Furanones or Liposomes, Targetting the irradiation of extra cellular polymeric biomolecules by enzymes [63] etc. Other strategies include disinfection of the insertion sites [64], surgical site irrigation, with biocides or antimicrobial locks to reduce indwelling catheter associated infections [65]. Cartin and Donlan have reported the ability of bacteriophage to degrade biofilm formation by *Staphylococcus epidermidis* [66]. Vejborg and Klemm have reported blocking of bacterial biofilm formation by a fish protein coating [67]. In dentistry, other than sodium hypochlorite irrigation, the newer techniques for biofilm eradication include ultrasonic irrigation, Ozone, plasma dental probe, photoactive disinfection with low energy LASER etc [32].

To prevent biofilm formation, the physical approaches like the use of low strength electrical field [68], electromagnetic field or ultrasound along with antibiotic therapy [69] are also very promising. A novel treatment based on disruption of quorum sensing system to inhibit biofilm formation has also been suggested by many workers [70]. Even the workers have suggested the inhibition of transcription of genes that are activated or repressed during initial biofilm formation will also help to prevent persistent infection due to biofilms. All these control strategies are on experimental basis and are not applicable for medical devices and have their own limitations to be used currently in patients.

To conclude, we must say biofilm develops slowly but has a major impact both clinically and economically on overall outcome of the patients treatment. The authors feel that, EARLY DETECTION AND NEWER TREATMENT OPTIONS FOR BIOFILM ASSOCIATED INFECTIONS ARE NEED OF THE HOUR.

Author details

Silpi Basak*, Monali N. Rajurkar, Ruchita O. Attal and Sanjay Kumar Mallick

Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (M.S.), India

References

- [1] Doulam RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant micro-organisms. *Clin Microbiol Rev.* 2002; 15(2): 167-193.
- [2] Davis DG, Geesy GG. Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continuous culture. *Appl Environ Microbiol.* 1995; 61: 860-7.
- [3] Introduction to Biofilms: Negative and positive impacts of biofilm. Available from: <http://www.cs.montana.edu/ross/personal/intro-biofilms-s3.html>.
- [4] Schopf JW, Hayes JM, Walter MR. Evolution on earth's earliest ecosystems: recent progress and unsolved problems. In: Schopf JW Ed, *Earth's earliest biosphere*. Princeton University Press, New Jersey, 1983; 15:143-147.
- [5] Jones DA, Amy PS. A thermodynamic interpretation of microbiologically influenced corrosion. *Corros* 2002;58:638-645.
- [6] Toole GO, Kaplan HB, Kolter R. Biofilm formation as microbial development. *Annu Rev Microbiol.* 2000; 54:49-79.

- [7] Geesey GG, Richardson WT, Yeomans HG, Irvin RT, Costerton JW. Microscopic examination of natural sessile bacterial populations from an alpine stream. *Can.J.Microbiol.* 1977;23(12): 1733-1736.
- [8] Henrici AT. Studies of fresh water bacteria. A direct microscopic technique. *J.Bacteriol.* 1933;25: 277-287.
- [9] Stoodley P, Sauer K, Davies D G, Costerton J W. Biofilms as complex differentiated communities. *Annu Rev Microbiol.* 2002; 56: 187: 209.
- [10] Carpentier B, Cerf O. Biofilms and their consequences, with particular reference to hygiene in food industry. *J Appl Bacteriol.* 1993; 75: 499-511.
- [11] An. YH, Dickinson RB and Doyle RJ. Mechanism of bacterial adhesion and pathogenesis of implant and tissue infections, In: Anand YH and Fridman RJ editors. *Handbook of bacterial adhesions: Principles, methods and applications.* Humana press; Totowa. NJ: 2000. P: 1-27.
- [12] Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J Bacteriol* 2002; 184: 1140-54.
- [13] Miller MB, Bassler BL. Quorum sensing in bacteria. *Ann. Rev. Microbiol.* 2001;55:165-199.
- [14] Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol* 2001; 55: 165-99.
- [15] Bassler B L, Small talk. Cell-to-cell communication in bacteria. *Cell* 2002; 109: 421-4.
- [16] Schafer AL. Generation of Cell-to-cell signals in quorum sensing: acyl homo serine lactone synthase activity of a purified *Vibrio fischeri* Lux I protein. *Proc Natl Acad Sci. USA* 1996; 93: 9505-9.
- [17] Schauder S, Shokat K, Surette MG, Bassler BL. The Lux S family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol* 2001; 41: 463-76.
- [18] Prouty AM. Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. *Infect Immun* 2002; 70: 2640-9.
- [19] Dunne WH Jr. Bacterial adhesion: seen any good biofilm lately. *Clin Microbiol Rev.* 2002 Apr: 155-166.
- [20] Lawrence JR, Korber DR, Hoyle BD, Costerton JW, Caldwell DE. Optical sectioning of microbial biofilms. *J Bacteriol.* 1991: 173:6558-67.
- [21] Flemming HC. Biofilms and environmental protection. *Water Sci Technol.* 1993; 27:1-10.

- [22] Baillie GS, Douglas LJ. Role of dimorphism in the development of *Candida albicans* biofilms. *J Med Microbiol* 1991; 48: 671-79.
- [23] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; 284:1318-22.
- [24] Mahenthiralingam E, Campbell ME, Speert DP. Non motility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. *Infect Immun* 1994; 62: 596-605.
- [25] Parsek MR, Singh PK. Bacterial biofilm: An emerging link to disease pathogenesis. *Ann Rev Microbiol.* 2003; 57: 677-701.
- [26] Nickel JC, Olson M, McLean RJ, Grant SK, Costerton JW. An ecological study of infected urinary stone genesis in animal model. *Br J Urol.* 1987; 59: 21-30.
- [27] Durack DT. Experimental bacterial endocarditis. IV. Structure and evolution of very early lesion. *J Pathol.* 1975; 115: 81-9.
- [28] Koch C, Hoiby N. Pathogenesis of cystic fibrosis. *Lancet* 1993; 341: 1065-9.
- [29] Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia Cepacia*. *Microbiol Rev.* 1996; 60 (3): 539-74.
- [30] Marsh D. Microbiological ecology of dental plaque and its significance in health and disease. *Adv Dent Res* 1994;8: 263-271.
- [31] Lamont RJ, Jenkinson HF. Life below gun line: pathogenic mechanism of *Porphyromonas gingivalis*. *Microbiol Mol Biol Rev.* 1998; 62: 1244-63.
- [32] Usha HL, Kaiwar A, Mehta D. Biofilm in endodontics: New understanding to an old Problem. *Int. journal of Contemporary Dentistry.* 2010; 1(3): 44-51.
- [33] Marrie TJ, Costerton JW. Mode of growth of bacterial pathogens in chronic polymicrobial human osteomyelitis. *J Clin Microbiol.* 1985; 22: 924-33.
- [34] Nickel JC, Costerton JW. Bacterial localization in antibiotic-refractory chronic bacterial prostatitis. *Prostate* 1993; 23: 107-114.
- [35] Dart JKG. Contact lens and prosthesis infections. In Tasman W and Jager EA editors. *Duane's foundation of Clinical ophthalmology.* Lippincott-Raven, Pa: Philadelphia: 1996; p: 1-30.
- [36] McLaughlin-Borlace L, Stapleton F, Matheson M, Dart JKG. Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. *J Appl Microbiol.* 1998;84: 827-38.
- [37] Hancock EW. Artificial valve disease. In: Schtant RC, Alexander RW, Rourke RAO Roberts R and Sonnenblick EH, editors. *The heart artiers and veins*, 8th ed, vol. 2. New York: McGraw-Hill; NY. 1994. p:1539-45.

- [38] Douglas JK, Cobbs CG. Prosthetic valve endocarditis In: Kaye D, editor. Infective endocarditis, 2nd ed. New York: Raven Press Ltd; N.Y. 1992. p: 375-396.
- [39] Cramton SE, Gerke C, Schll NF, Nicholas WW, Gotz F. The intercellular adhesion (ica locus) is present in *Staphylococcus aureus* and is required for biofilm formation. Infect Immun. 1999; 67:5427-33.
- [40] Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Gotz F. Molecular basis of intercellular adhesions in the biofilm forming *Staphylococcus epidermidis*. Mol Microbiol. 1996; 20: 1083-91.
- [41] National Nosocomial Infections Surveillance (NNIS) System Report. Data summary from January 1992 through June2004. Am J Infect Control 2004; 32; 470-485.
- [42] Warren JW. The catheter and urinary tract infection. Med Clin North Am 1991; 75:481 - 493.
- [43] Darouiche R, Device associated infections : A macroproblem that starts with micro-adherence. Clin. Infect Dis. 2001; 33: 1567-1572.
- [44] Traunter BW and Darouiche RO. Catheter –associated infections: Pathogenesis affects Prevention. Arch Intern Med, 2004; 164: 842-850 .
- [45] Lachman P and Yuen S. Using care bundles to prevent infection in neonatal and paediatric .ICUs. Curr. Opin infect Dis, 2009; 22: 224-228.
- [46] Fiel S. Guidelines and critical pathways for severe hospital- acquired pneumonia, Chest. 2011;119:412-418.
- [47] Levis K. riddle of biofilm resistance. Antimicrob Agents Chemother 2001;45(4) : 999-1007.
- [48] Brooun a, Liu S, Lewis K. A dose- response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2000; 44; 640-646.
- [49] Molin S, Tolker-Nielson T. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilization of the biofilm structure. Curr Opin Biotechnol. 2003;14(3): 255-256.
- [50] Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom intractions: *Candida alicans* and bacteria. FEMS Microbiol Lett. 2009; June:1-8.
- [51] Peters BM, Jabra-Rizk MA, Scheper MA et al. Microbial interactions and differential protein expression in *Staphylococcus aureus* and *Candida albicans* dual-species biofilms. FEMS Imm Med Microbiol 2010; 59: 493-503.
- [52] Harriott MM, Noverr MC. *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrob Agents Chemother. 2009; 53(9): 3914-3922.

- [53] Hogan DA, Kolter R. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. *Science*. 2002; 296(5576): 2229-2232.
- [54] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; 284:1318-1322.
- [55] Washington CW Jr, Stephen DA, William MJ, Elmer WK, Gray WP, Paul CS, Gail LW. In Koneman's Colour Atlas and Textbook Of Diagnostic Microbiology, 6th ed, Lippincott Williams & Wilkins, Philadelphia PA, USA, 2006.
- [56] Merritt JN, Kadouri DE, Toole GO. Grouping and analysing static biofilms. Ch Basic Protocol I, In current protocols in Microbiology, 2005; unit 1B. 1.1-1B.1.7
- [57] Stepanovi s, Vukovi D, Daki I, Savib, Svabi-Vlahovi (M). A modified micotiter-plate test for quantitation of staphylococcal biofilm formation. *J Microbiol. Methods*. 2000; 40:175- 179.
- [58] Bose S, Khodke M, Basak S, Mallick S K. Detection of biofilm producing *Staphylococci*: Need of the hour. *J. Clin and Diagnostic Res* [serial online] 2009 December [cited: 2009 December 7]; 3:1915-1920. Available from <http://www.jcdr.net/back.asp?issn=0973-709x&year=2009&month=December&volume=3&issue=6&page=1915-1920&id=469>.
- [59] Lee HW, Koh YM, Kim J et al Capacity of multidrug resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces *Clin. Microbiol Infect*. 2008;14:49-54.
- [60] norouzi F, Mansouri S, Moradi M, Razavi M Comparison of cell surface hydrophobicity and biofilm formation among ESBL and non ESBL producing *Pseudomonas aeruginosa* from clinical isolates. *African Journal of Microbiology Research* 2010;4(11):1143-1147.
- [61] Khan F, Shukla I, Rizvi M, Mansoor T, Sharma SC Detection of biofilm formation in *Staphylococcus aureus*. Does it have a role in treatment of MRSA infections? *Trends in Medical Research* 2011;6:116-123.
- [62] Spencer RC. Novel methods for prevention of infection of intravascular devices. *J Hosp Infect*. 1999; 43suppl: S127- 35.
- [63] Johansen C, Falholt P, Gram L. Enzymatic removal and disinfection of bacterial biofilm. *Appl Environ Microbiol*. 1997; 63: 3724-8.
- [64] Maki DG, Ringer M, Alvarado CJ Prospective randomized trial of povidone iodine, alcohol and chlorhexidine for prevention of infection associated with central venous and arterial catheters. *Lancet* 1991;338:339-343.
- [65] Curtin J, Cormican M, Fleming G, Keeiehan J, Colleran E Linezolid compared with eperezolid, vancomycin and gentamicin in an in vitro model of antimicrobial lock

therapy for *Staphylococcus epidermidis* central venous catheter related biofilm infections. *Antimicrobial. Agents Chemother.* 2003;47:3145-3148.

- [66] Curtin JJ and Donlan RM Using bacteriophages to reduce formation of catheter associated biofilms by *Staphylococcus epidermidis*. *Antimicrobial. Agents Chemother.* 2006;50(4):1268-1275.
- [67] Vejborg RM and Klemm P Blocking of bacterial biofilm formation by a fish protein coating *Appl. Environ. Microbiol.* 2008.
- [68] Blenkinsopp SA, Khoury AE, Costerton JW Electrical enhancement of biocide efficacy against *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* 1992;58:3770-3773.
- [69] Huang CT, James G, Pitt WG, Stewart PS Effects of ultrasonic treatment on the efficacy of gentamicin against established *Pseudomonas aeruginosa* biofilms. *Colloids Surfaces B Biointerfaces* 1996;6:235-242.
- [70] Hartman G, Wise R. Quorum sensing. Potential means of treating Gram negative infection? *Lancet* 1998; 351: 848-9.